US ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE
ABERDEEN PROVING GROUND, MARYLAND 21010-5425

USAMRICD-TR-89-08

ANALYTICAL METHOD DEVELOPMENT AND IN VIVO AND IN VITRO EXPOSURE STUDIES OF BIS-(TRIFLUOROMETHYL)DISULFIDE

Ming L. Shih
John R. Smith

June 1989

US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK, MARYLAND 21701-5012

DISPOSITION INSTRUCTIONS

Destroy this report when no longer needed. Do not return to the originator.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

In conducting the work described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp Date Jun 30, 1986

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) USAMRICD-TR-89-08	5. MONITORING ORGANIZATION REPORT NUMBER(S) USAMRICD-TR-89-08
6a. NAME OF PERFORMING ORGANIZATION US Army Medical Research Institute of Chemical Defense 6b. OFFICE SYMBOL (If applicable) SGRD-UV-VA	7a. NAME OF MONITORING ORGANIZATION US Army Medical Research Institute of Chemical Defense, SGRD-VU-RC
6c. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5425	7b. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5424
8a. NAME OF FUNDING/SPONSORING ORGANIZATION 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 61102A; PROJECT NO. 3M161102B; TASK NO. S11A; WORK UNIT ACCESSION NO. 389

11. TITLE (Include Security Classification)
Analytical Method Development and In Vivo and In Vitro Exposure Studies of Bis-(trifluoroethyl)disulfide

12. PERSONAL AUTHOR(S)
Shih, M.L.; Smith, J.R.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Technical	FROM Jun 88 TO Oct 88	June 1989	23

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	04/11		Bis-(trifluoromethyl)dislufide, Gas Chromatography, Electron capture, Binding, Partition Ratio, In Vivo, In Vitro
06	15		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

A GC/EC analytical method was developed to quantitate bis-(trifluoromethyl)disulfide accurately in exhaled air of exposed animals. Using the headspace sampling technique qualitative determination of unbound compound in biological sample can be achieved. Partition ratio in plasma was much higher than in water (4.26 vs. 0.14). In vitro studies indicated that irreversible binding and interaction could occur when bis-(trifluoromethyl)-disulfide was present in the blood. The unbound portion disappeared at a faster rate from RBC than from plasma. Bis-(trifluoromethyl)disulfide might have penetrated the lung tissue and entered the blood stream, but we did not detect any unbound compound in blood from exposed animals. The results could be explained by irreversible binding and sequestering action by blood components.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL Gerald P. Jaax, LTC, VC	22b. TELEPHONE (Include Area Code) (301) 671-3804 22c. OFFICE SYMBOL SGRD-UIV-V

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

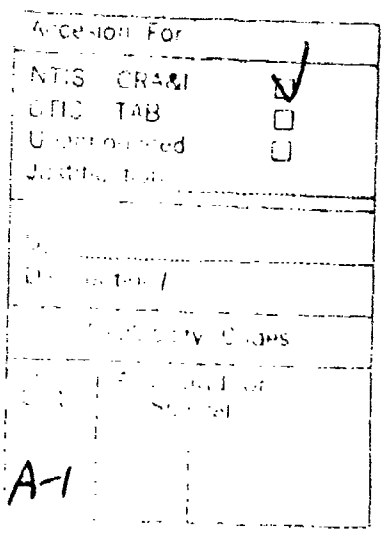
UNCLASSIFIED

PREFACE

The work described in this report was initiated in June 1988 and finished in October 1988 under protocol 1-03-87-000-A-422 at the US Army Medical Research Institute of Chemical Defense (USAMRICD) and was authorized under US-UK-CA memorandum of understanding on chemical-biological defense (MOU) dated June 1981. All laboratory data were recorded in laboratory notebook No. 040-88.

ACKNOWLEDGEMENT

The authors would like to express their sincere appreciations to LTC David T. Zolock for his valuable discussions and suggestions, Steve Musser and Edward M. Jakubowski for the mass spectrometry work, and Leonard Buettner for supplying the compound bis-(trifluoromethyl)disulfide.



LIST OF FIGURES

INTRODUCTION

The Analytical Chemistry Branch of USAMRICD was tasked to develop an analytical method for determining the presence of compound bis-(trifluoromethyl)disulfide in either exhaled air or blood samples taken from animals exposed to the compound. This organohalogen compound was first synthesized in 1959 (1). A pesticide action was reported in 1970 by Dear and Gilbert (2): the vapor of one pound per thousand cubic feet concentration caused 100% mortality in beetles, larvas, and larvae eggs. The chlorine analog was relatively nontoxic. The common biochemical mechanism which explains toxicities in all species has not been identified. This report presents an analytical method to quantitate the unbound compound in either biological matrices or exhaled air. The analysis results of blood samples from exposed animals as well as degradation phenomenon observed in in vitro exposure studies might lead to a better understanding of the biochemical basis of the toxicity of this compound. Partitions of the compound in water and blood were also studied.

MATERIALS AND METHODS

We selected the headspace sampling technique and the gas chromatograph instrument using an electron-capture detector (GC/EC) as the analytical method because of the volatility and the characteristic halogen functional groups of the compound. Flame photometric and mass detectors were also used when necessary for structure confirmation. The headspace sampling technique is an established technique for quantitating volatile compounds in biological matrices. This technique eliminates the need for an exhaustive sample extraction procedure because volatile compounds can be driven off the biological matrix when samples are heated and air space above the matrix can be sampled and injected directly onto the GC column.

Material

A Hewlett Packard gas chromatograph model 5880 equipped with an electron capture detector was used. A GS-Q megabore column (30 m x 0.53 mm I.D.) installed in the GC oven was maintained at an isothermal temperature of 120°C and the helium carrier gas had a flow rate of 10 ml/min. The injector temperature was off and detector temperature was set at 250°C using argon/methane as makeup gas at a flow of 20 ml/min. The Finnigan mass spectrometer model 4500 was operated in chemical ionization mode and same GC conditions were used.

Gaseous bis-(trifluoromethyl)disulfide was obtained from PCR Research Chemicals (Gainesville, FL) in gas cylinder. The liquid form used in these studies was made by cooling the vapor transferred from the cylinder. A 100% purity was stated in the company data sheet, but we identified about 6% impurities including carbon disulfide, carbonyl sulfide, and chloroform.

Procedure

1. GC/EC Calibration Curve for Air Sample

The response versus concentration calibration curves for air samples using GC/EC detection system were established as follows. One liter calibrated gas sampling bags (Calibrated Instruments Inc., Ardsley, N.Y. 10502) were inflated with a pulse pump (same source). Different concentrations of bis-(trifluoromethyl)disulfide solutions were prepared in acetonitrile and 10 uL of each standard solution was added to the gas bag. The final concentrations of bis-(trifluoromethyl)disulfide in the gas bags were 13.4, 28.9, and 48.6, and 152.0 ug/L. Five microliters of the air sample was injected onto the GC column.

2. GC/EC Calibration Curve for Aqueous and Plasma Sample Using Headspace Sampling Technique

To spike water and plasma samples the neat bis-(trifluoromethyl)-disulfide was dissolved in acetonitrile at 41 ug/mL concentration and various microliter volumes of this stock solution were spiked to 1 mL of water or sheep plasma to prepare standard solutions of concentrations ranging from 82 to 820 ng/mL. Each spiked standard solution was kept in a two mL GC autosampler glass vial that was crimp-sealed with a teflon disc and metal cap. The vials were placed in a constant temperature water bath maintained at 37°C. The standard solutions were incubated immediately following spiking. Two microliters of the headspace was sampled and injected into the GC at 5 and 15 minutes incubation time.

3. Rat Intraperitoneal Absorption Studies

Three groups of four rats each were injected intraperitoneally with neat bis-(trifluoromethyl)disulfide liquid at doses of 0.5, 5, and 50 uL and beheaded 1-4 hours after exposure. Each group of rats was kept in a closed chamber, and the exhaled air was pumped into one liter gas sampling bag for analysis. Blood samples were collected in rubber stoppered serum tubes and centrifuged immediately in a refrigerated centrifuge (10°C) to separate plasma from red blood cells. One mL of whole blood, plasma, or red blood cells was placed in a two mL GC autosampler glass vial sealed and incubated as above. Fifty uL of the air above the sample was injected into the gas chromatograph.

4. Sheep Inhalation Studies

Sheep were exposed to bis-(trifluoromethyl)disulfide at 5 ppm concentration in breathing air for 10 minutes. Blood samples were drawn and centrifuged immediately in a refrigerated centrifuge to separate plasma from red blood cells and analyzed as above.

5. Partition Ratio in Water

The partition ratio of bis-(trifluoromethyl)disulfide in water was determined by adding 10 uL of the neat compound to 50 mL water in a 60 mL

serum vial. The vial was crimp-sealed and contents were stirred with a small magnetic stirrer for 30 minutes at room temperature. Equal injection volumes, 0.6 uL of liquid phase or air phase, were injected into GC. The peak areas of water phase versus air phase were used to derive the partition ratio of this compound in water.

6. In Vitro Exposure Studies

Bis-(trifluoromethyl)disulfide was dissolved in acetonitrile at a concentration of 7.56 mg/mL. Ten microliters of the solution was added to 1 mL of sheep plasma or red blood cell to achieve a final concentration of 75.6 ug/mL and incubated at 37°C. Water was used as a control. One uL volume of headspace was taken periodically and analyzed over a five-hour time period.

RESULTS AND DISCUSSION

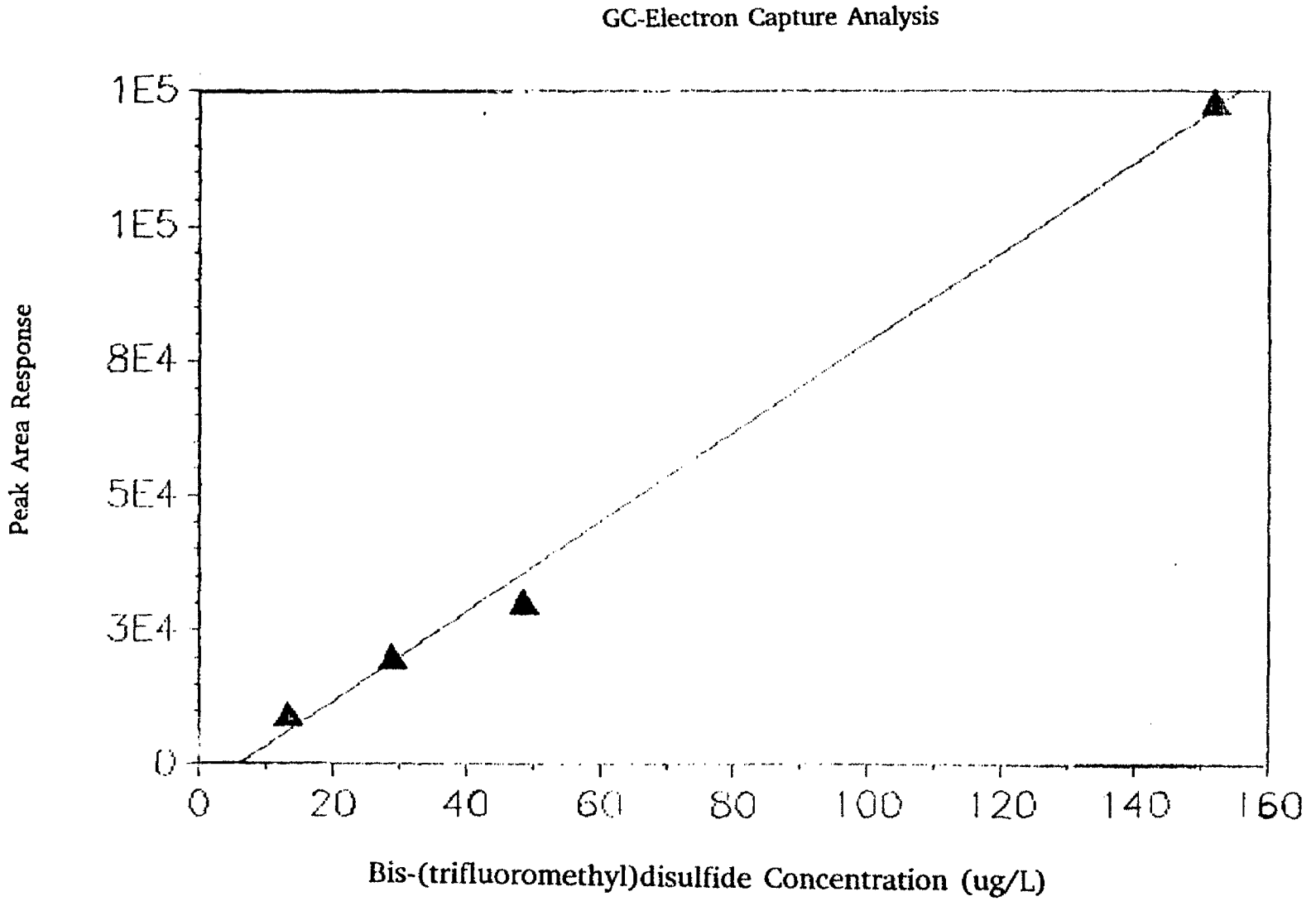
1. Standard Calibration Curve for Air Sample

Table 1 lists the linearity, intra- and inter-day repeatabilities of the detector response. The values of the correlation coefficient of the calibration curve were determined on three different days and were not less than 0.997. The coefficient of variation (CV; standard deviation over mean) for repetitive injections ranged from 1.7 to 7.9%. The averaged regression line for the calibration curve was plotted in Figure 1 with a slope and intercept values of 833.4 and -4898 respectively.

Table 1
Linear Calibration Curve for Air Sample

	Peak Area Count											
	Day 1			Day 2			Day 3			Inter-Day		
Conc(ug/L)	Mean	CV%	n	Mean	CV%	n	Mean	CV%	n	Mean	CV%	n
13.4	9573	7.9	6	9755	1.8	3	8895	7.3	3	9407	4.8	3
28.9	20145	6.4	6	20402	3.1	3	19830	4.0	3	20125	1.4	3
48.6	29688	2.7	6	31760	5.2	3	29424	5.9	3	30290	4.2	3
152.0	121146	1.7	6	121737	2.7	3	126235	2.6	3	123039	2.2	3
r^2	0.997			0.998			0.997			0.997		
slope	818.6			819.6			862.1			833.4		
intercept	-4575			-3862			-6256			-4898		

Figure 1. Standard Calibration Curve for Air Sample



2. Standard Calibration Curve for Aqueous and Plasma Samples Using Headspace Sampling Technique

Table 2 presents the peak area response versus concentration at 5 and 15 minute incubation times from aqueous or plasma samples using the headspace sampling technique.

Table 2
Linear Calibration Curve for Aqueous and Plasma Sample

	Peak Area Counts				
	5 minutes			15 minutes	
Conc (ng/ml)	H_2O	Plasma	%*	H_2O	Plasma
82	16273	ND**		17725	ND
123	26277	420	2	22573	ND
164	33778	7376	22	30466	ND
287	60258	12929	21	54538	ND
410	74136	-		80946	ND
615	106174	16682	16	103758	ND
820	152446	23916	16	147585	ND
r^2	0.997	0.926		0.997	
slope	176	26.8		174	
intercept	4083	1815		3190	

* Percent peak area count in the headspace of plasma versus H_2O ** ND stands for not detectable

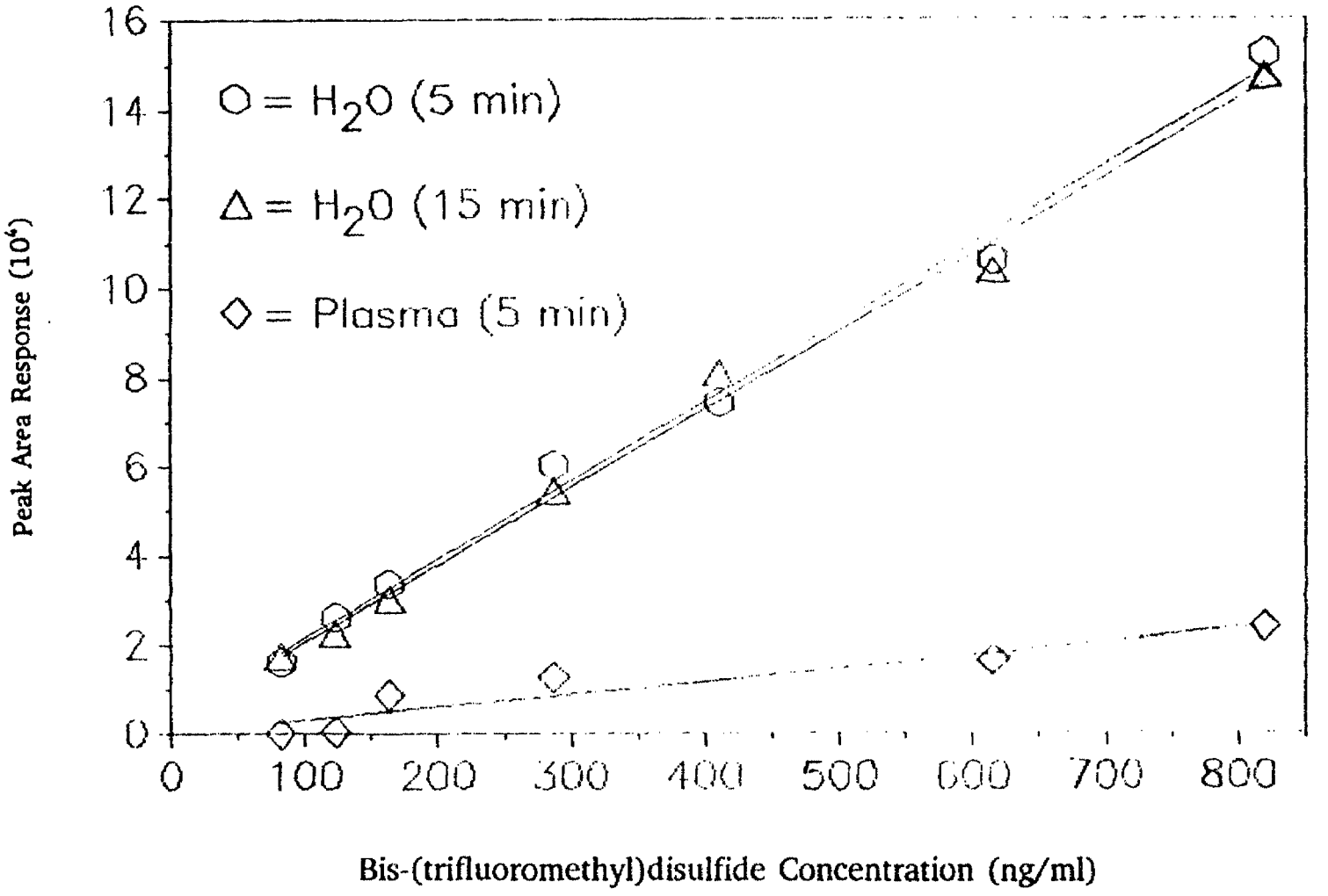
The linear regression plot of detector response versus concentrations of spiked water and plasma samples using headspace analysis is shown in Figure 2.

Bis-(trifluoromethyl)disulfide was very easily and completely driven into the head space from the water solutions. This was proven when the aqueous phase was injected on the column and no compound could be detected in the aqueous phase after the sample was heated. Reproducible slopes were observed for the 5- and 15- minute plots for the water samples, and correlation coefficients for the linear regression line (r^2) were 0.997. In contrast the correlation coefficient for the plasma samples at 5-minute incubation time was poor (0.926). At 15 minutes no free compound could be detected in the headspace of any plasma samples.

As seen in Table 2 the percent peak area count of the plasma headspace sample was negligible at low concentrations. The percent maintained between 16 to 21% (average value 19% of total spiked amount) between the concentration 164 to 820 ng/mL. This seems to indicate that bis-

Figure 2. Standard Calibration Curve for Aqueous and Plasma Sample

Samples Heated at 37°C



(trifluoromethyl)disulfide was irreversibly bound to plasma initially and hence very little released to the headspace. At higher concentrations both binding and partitioning processes were involved. The average percent of bis-(trifluoromethyl)disulfide remaining in plasma was 81% from 164-820 ng/mL. This equated to a partition ratio of 4.26 in plasma. The absence of free compound in the headspace at 15 minutes indicated that some sequestering interaction other than binding occurred between the compound and plasma components during this time period.

3. Partition Ratio of Bis-(trifluoromethyl)disulfide in Water

Table 3 shows the peak area response of repetitive injections of an equal volume of the air phase and water phase. The partition ratio of bis-(trifluoromethyl)disulfide in water was found to be 0.14 by dividing the average peak area of water over air.

Table 3
Partition Ratio of Bis-(trifluoromethyl)disulfide between Air and Water

	Peak Area Count	
	Aqueous Phase	Air Phase
Average	1101188	7994186
SD	63502	1511775
CV	5.8%	14.4%
No. of Injection	4	5

Partition Ratio = 1101188/7994186 = 0.14

The partition ratio is defined as the equilibrium ratio between the concentration in water versus in air as in equation (1).

Partition Ratio = Concentration in water/Concentration in air (1)

The concentration in water is the same as the solubility in water (S_{water}). The volumes occupied by the water and air are 50 and 10 mL respectively. The density of the compound is 1.52 and 10 uL was added. The amount present in the air would be the total amount added minus the amount dissolved in the water as expressed in equation (2).

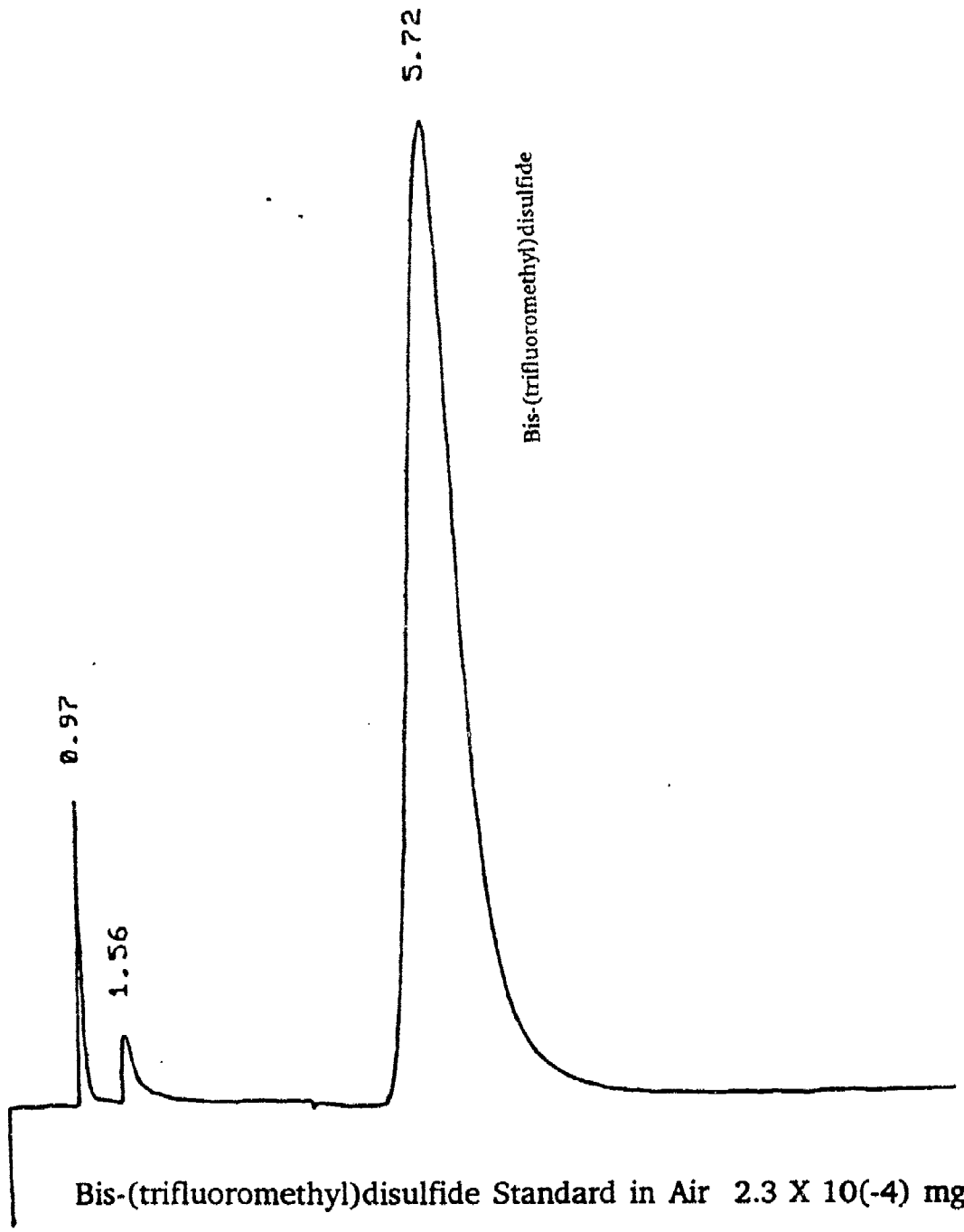
Amount in air = 1.52 (mg/uL) x 10 uL - S_{water} (mg/mL) x 50 mL (2)

And the concentration in air can be expressed as the amount in air (equation 2) over the volume of the air space.

Concentration in Air (mg/mL) = (15.2 - S_{water} x 50)/10 (3)

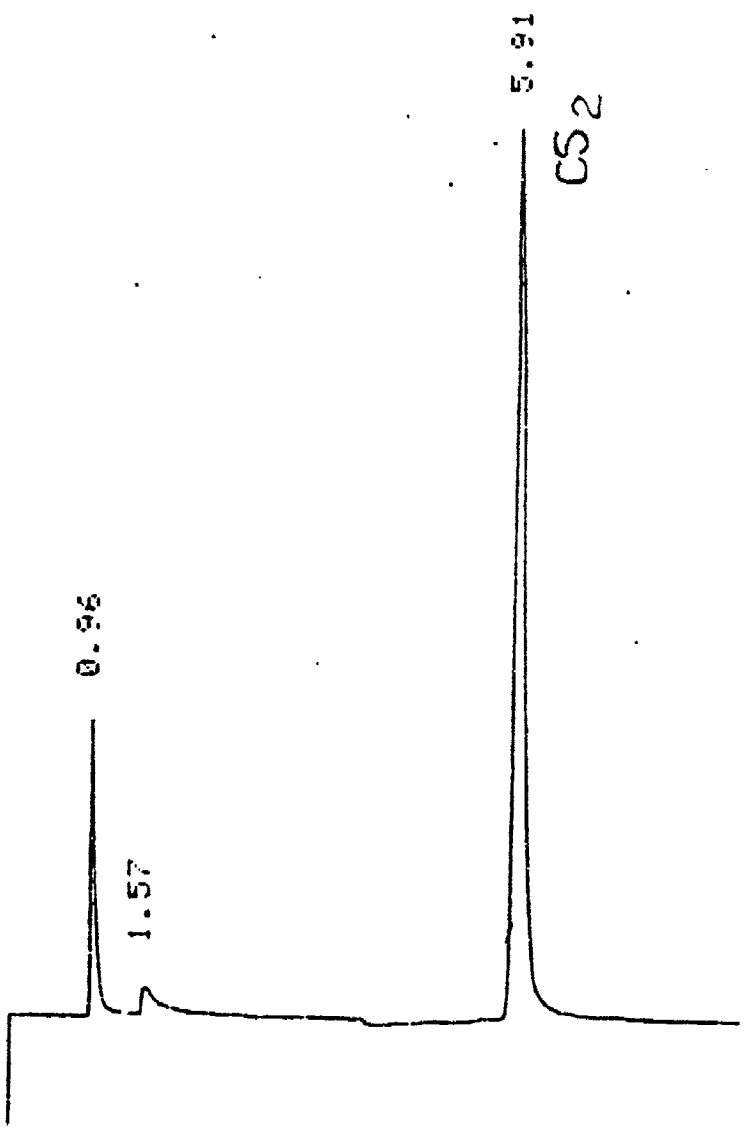
Therefore, equation (1) can be rewritten as equation (4):

Figure 3. GC/EC Chromatogram of Bis-(trifluoromethyl)disulfide



Bis-(trifluoromethyl)disulfide Standard in Air 2.3 X 10(-4) mg/ml

Figure 4. GC/EC Chromatogram of CS_2



$$\text{Partition ratio} = 0.14 = S_{water}/((15.2 - S_{water} \times 50)/10) \quad (4)$$

By solving the above equation the solubility in water was calculated to be 0.12 mg/mL.

We found, however, that a definitive solubility value in water was difficult to assess because the compound was in constant dynamic equilibrium between the air and water phases and had a tendancy to escape to the air phase. If a solution of bis-(trifluoromethyl)disulfide was left open, very little compound could be detected. In a closed system when partition equilibrium was reached the solubility in water would be varied with the concentration in the air space. Therefore, the partition ratio would be a more meaningful constant.

4. Analysis of Blood Samples and Exhaled Air from Rat Injected IP

Figures 3 and 4 show that the retention times of bis-(trifluoromethyl)disulfide and CS_2 are 5.72 and 5.91 minutes respectively in the GC/EC chromatograms. The peak at 5.16 minutes seen in the chromatogram of control blood (Figure 5) was not a sulfur compound as confirmed by flame photometric detector, but structural determination was not amenable for mass spectrometer identification. In a spiked blood sample this unidentified peak and CS_2 peak were buried under bis-(trifluoromethyl)disulfide and appeared as only one single peak (Figure 6) and reappeared when bis-(trifluoromethyl)disulfide was sequestered after 91 minutes incubation (Figure 7). Headspace analyses of all the blood samples from exposed rats did not show the presence of bis-(trifluoromethyl)disulfide at the retention time of 5.7 minutes (Figures 8), but COS and CS_2 peaks were present. Trace amount of CS_2 and COS were also detected in the exhaled air by EC. Approximately 1 ug/mL of CS_2 detected in the blood. Therefore, it probably was an impurity of the neat material and not a biotransformed metabolite.

5. Analysis of Blood Samples from Sheep Inhalation Studies

In the sheep inhalation study we did not detect any parent compound in the head space of plasma, RBC, or whole blood samples. In the expired air samples small amounts of CS_2 and COS were detected using mass spectrometric techniques.

6. In Vitro Exposure Studies

The observation of irreversible binding and fast elimination of unbound bis-(trifluoromethyl)disulfide in plasma led us to use a system of much higher concentration to observe the kinetics over a longer period of time. The time course of disappearance of bis-(trifluoromethyl)disulfide from the headspace is shown in Figure 8 at a semi-logarithm scale. The same concentration in water was used as the control. In the water control a constant level of compound was present in the headspace, indicating no leakage in the system or the possible reaction between bis-(trifluoromethyl)disulfide and water. In the plasma samples the unbound compound disappeared initially at a slower rate and then a faster one over

Figure 5. GC/EC Chromatogram of Headspace of Control RBC

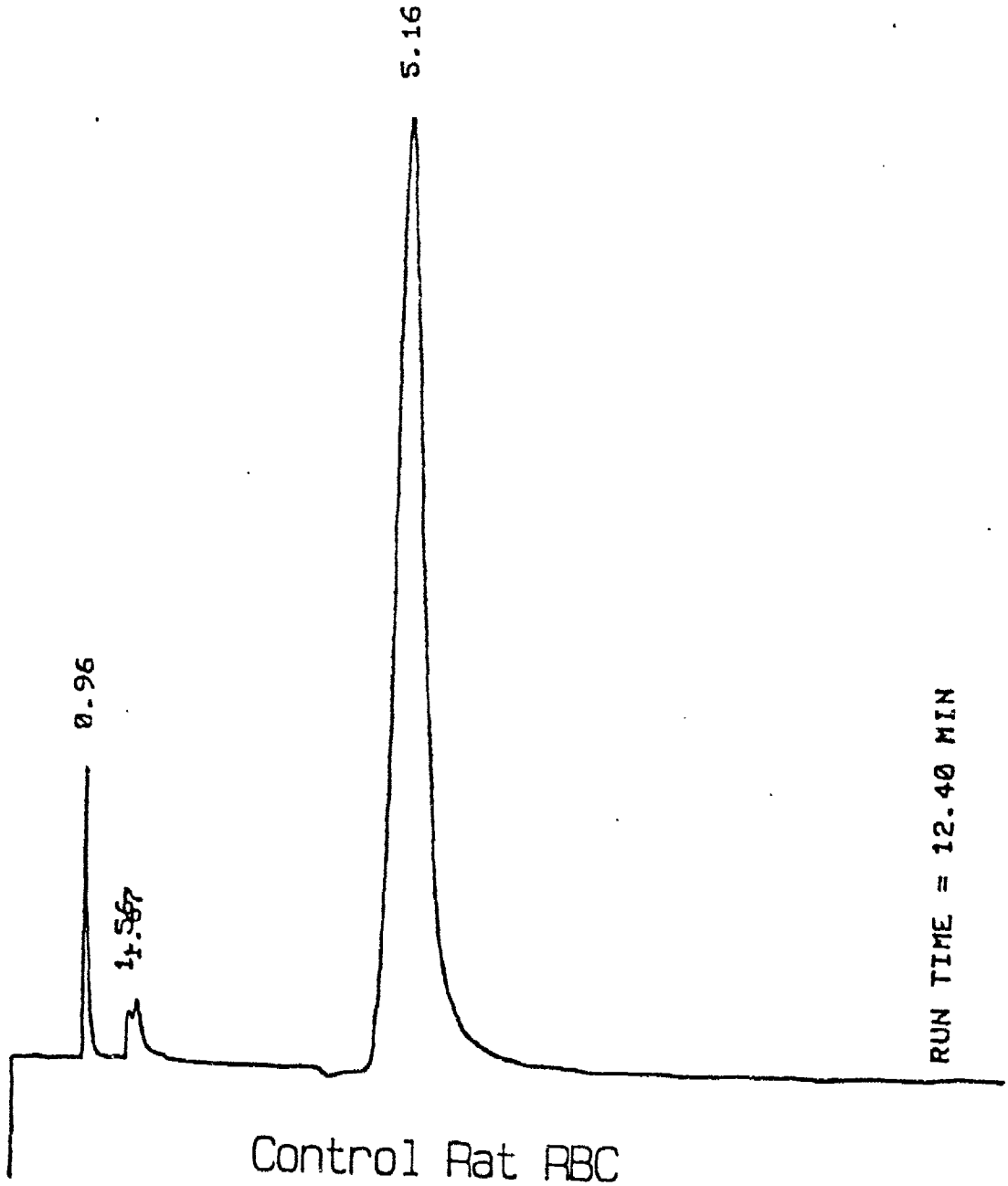
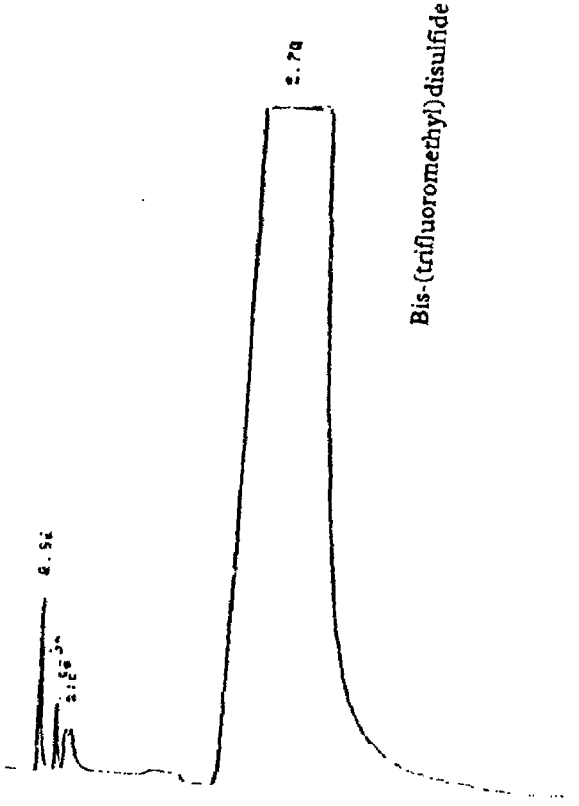
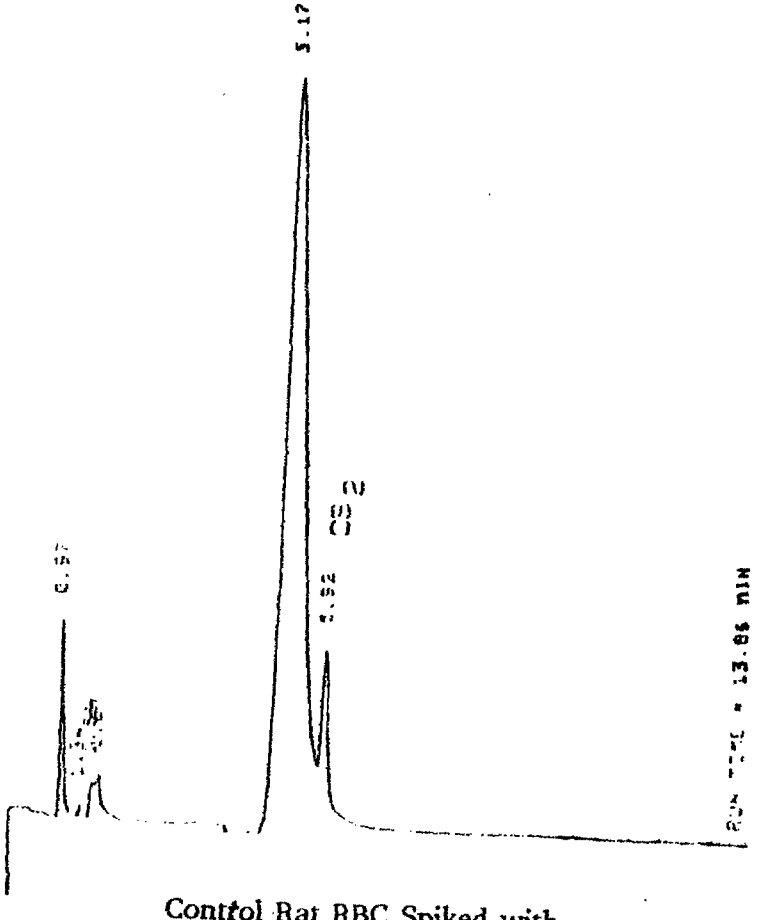


Figure 6. GC/EC Chromatogram of Headspace of RBC Spiked with Bis-(trifluoromethyl)disulfide



Control Rat RBC Spiked with
Bis-(trifluoromethyl)disulfide
9.1 x 10 (-3) mg/ml
45 min. after addition



Control Rat RBC Spiked with
Bis-(trifluoromethyl)disulfide
9.1 x 10(-3) mg/ml
90 min. after addition

Figure 7. GC/EC Chromatogram of Headspace of RBC from Rats Injected Intraperitoneally with Bis-(trifluoromethyl)disulfide

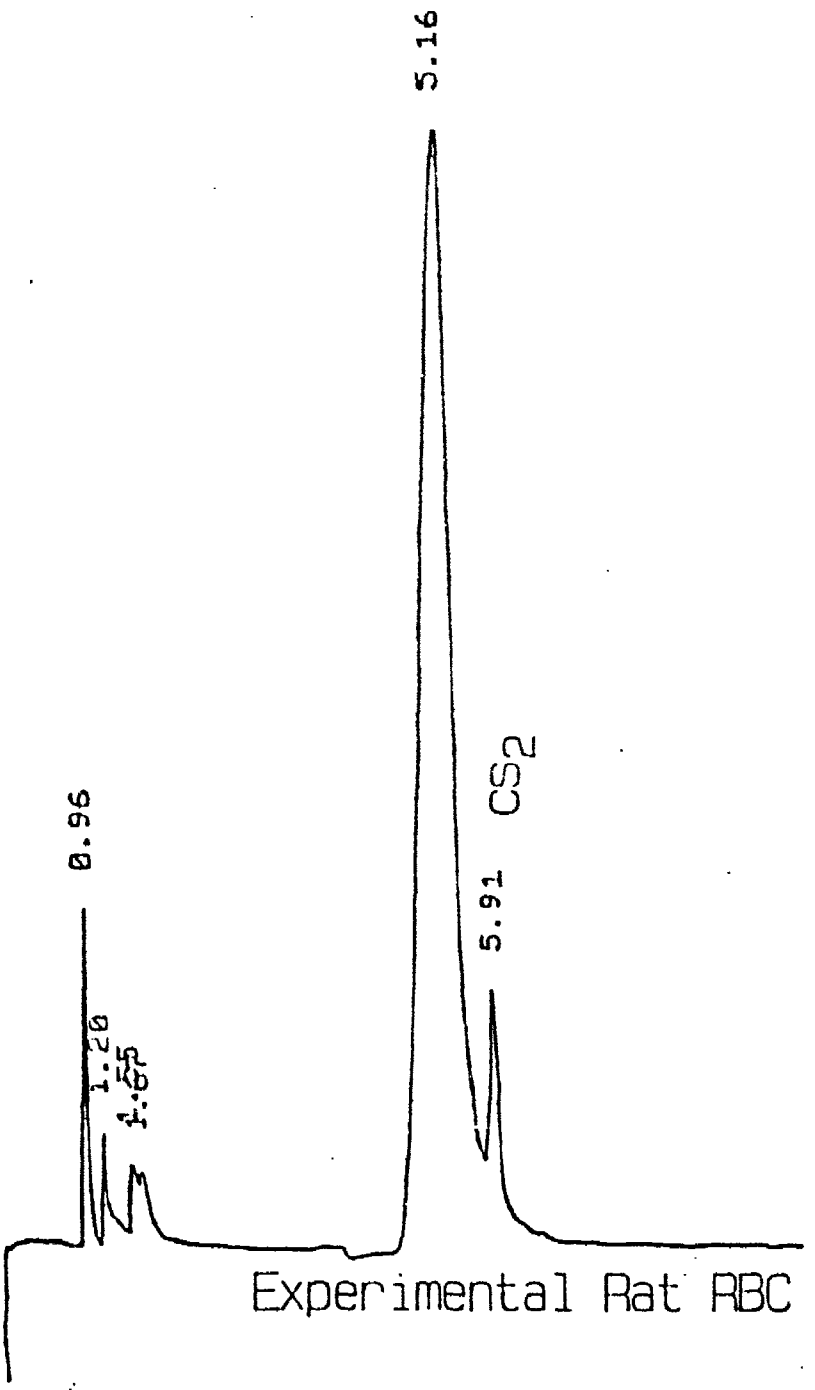
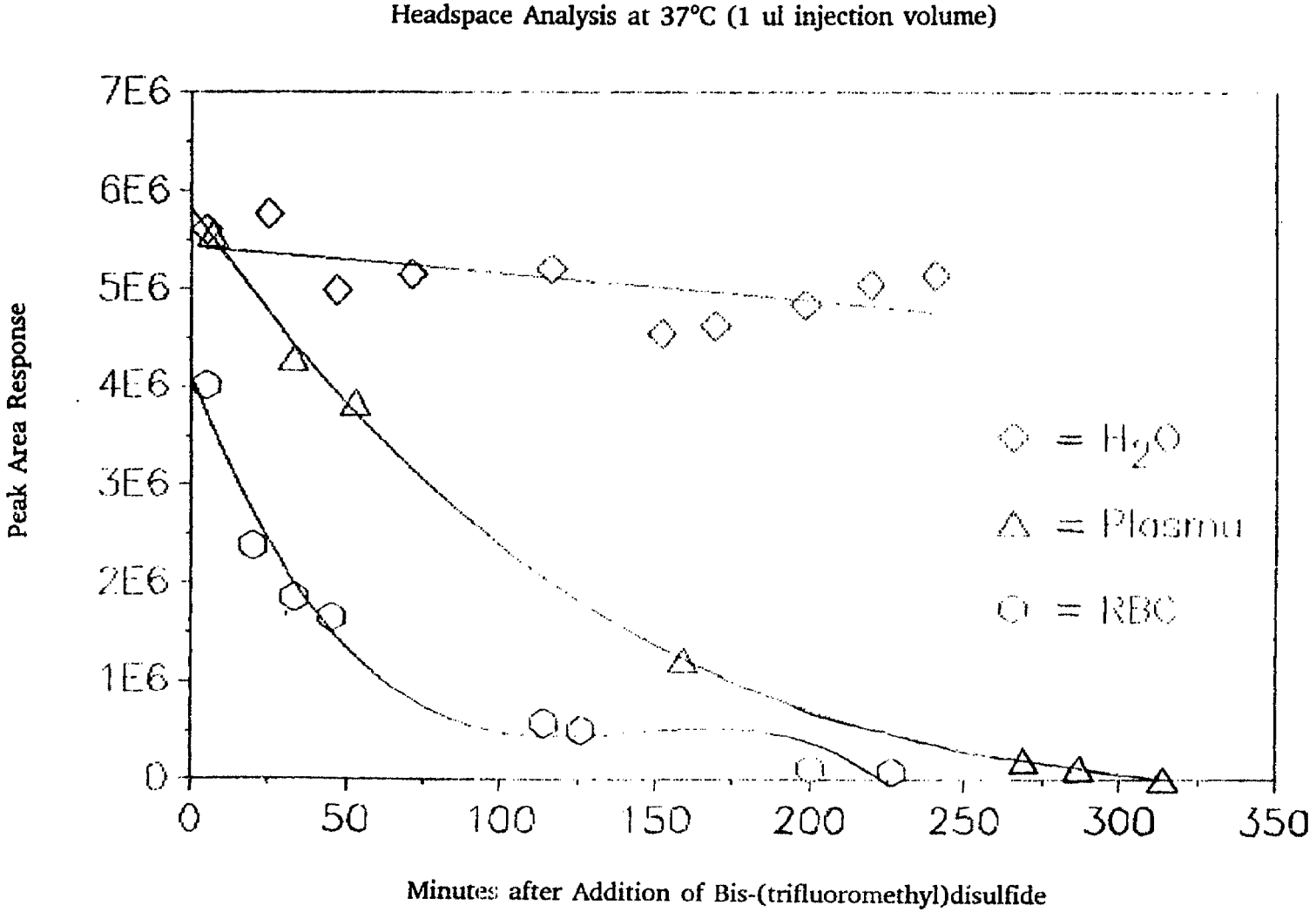


Figure 8. In Vitro Exposure Study of RBC and Plasma to Bis-(trifluoromethyl)disulfide



the five-hour period. Similar biphasic kinetics were observed in RBC samples, and rates in RBC were faster than in plasma.

In the same sheep exposure study results obtained using an infra-red technique indicated that about 27% of a given dose was retained by sheep and only 73% was recovered in the exhaled air (3). Pathological studies indicated damage in endothelial and red blood cell membranes (4). Our data on blood samples from exposed animals did not indicate the presence of unbound compound. But the in vitro studies strongly support the possibility that the compound could penetrate through lung tissue as well as interact with, and be sequestered by, blood components without being detected through headspace analysis.

CONCLUSIONS

This report demonstrates that a gas chromatography/electron capture method can reproducibly quantitate exhaled air samples or aqueous samples when the headspace sampling technique is used. The lower detection limit is 13 ppb in air sample or 70 pg on column injection. For blood samples only qualitative determination of unbound compound can be made because of the irreversible binding between blood and the compound and degradation of bis-(trifluoromethyl)disulfide when the samples are aged.

The partition ratio of bis-(trifluoromethyl)disulfide was 0.14 in water and 4.26 in plasma.

In vitro exposure studies indicated the occurrence of irreversible binding and biphasic degradation of bis-(trifluoromethyl)disulfide in plasma and red blood cells.

In animal exposure studies we could not detect any unbound compound in the headspace of either blood samples from i.p. dosed rats and from sheep exposed through inhalation or exhaled air from the dosed rats. CS_2 and COS were detected in the exhaled air of exposed animals. We believe they were from the impurities present in the original compound and not the result of biotransformation of the parent compound because the amount detected was at a trace level compared to the dose given and because the impurities were also present in spiked water samples. No other volatile chemical decomposition products were observed during the exposure period.

REFERENCES

1. Tullock, C. W. US Patent 2,884,453, "Reaction of Metal Fluorides with Thiophosgene and Perchloromethyl Mercaptan," Apr. 28, 1959.

2. Dear, R.E.A., Gilbert, E.E., US Patent 3,505,459, "Fumigation with Bis(trifluoromethyl) Disulfide," Apr. 7, 1970.

3. Braue, E.H., USAMRICD, personal communication.

4. Petrali, J.P., USAMRICD, personal communication.

Distribution List

Addresses	Copies
Defense Technical Information Center ATTN: DTIC-DDAC Cameron Station, Bldg 5 Alexandria, VA 22314-6145	12
Commander US Army Medical Research and Development Command Fort Detrick, MD 21701-5012	2
HQDA(DASG-HCD) Washington, DC 20310	1
Director Walter Reed Army Institute of Research Bldg 40 Washington, DC 20307-5100	1
Commander Letterman Army Institute of Research Bldg 1110 Presidio of San Francisco, CA 94129-6800	1
Commander US Army Aeromedical Research Laboratory ATTN: Scientific Information Ctr P.O. Box 577 Fort Rucker, AL 36362-5000	1
Commander US Army Biomedical Research and Development Laboratory Bldg 568 Fort Detrick, MD 21701-5010	1
Commander US Army Medical Research Institute of Infectious Disease Bldg 1425 Fort Detrick, MD 21701-5011	1
Commander US Army Research Institute of Environmental Medicine Bldg 42 Natick, MA 01760-5007	1
Commandant US Army Chemical School ATTN: ATZN-CM-C Fort McClellan, AL 36205	1
Director Armed Forces Medical Intelligence Center Fort Detrick, MD 21701-5004	1
Commander US Army Institute of Dental Research Bldg 40 Washington, DC 20307-5100	1
Commander US Army Institute of Surgical Research Bldg 2653 Fort Sam Houston, TX 78234-6200	1
Commandant Academy of Health Sciences US Army ATTN: HSHA-CDC Fort Sam Houston, TX 78234-6100	1
Commandant Academy of Health Sciences US Army ATTN: HSHA-CDM Fort Sam Houston, TX 78234-6100	1
Mr Thomas R. Dashiell Director, Environmental and Life Sciences Office of the Deputy Under Secretary of Defense (Rsch & Adv Technology) Room 3D129 Washington, DC 20301-2300	1

Commander
US Army Training and Doctrine Command
ATTN: ATMD
Fort Monroe, VA 23651 — 1

Commander
US Army Nuclear and Chemical Agency
7500 Backlick Road
Bldg 2073
Springfield, VA 22150-3198 — 1

Biological Science Division
Office of Naval Research
Arlington, VA 22217 — 1

Executive Officer
Naval Medical Research Institute
Naval Medicine Command
National Capital Region
Bethesda, MD 20814 — 1

USAF School of Aerospace Medicine/VN
Crew Technology Division
Brooks AFB, TX 78235-5000 — 1

Commander
US Army Medical Research Institute of Chemical Defense
ATTN: SGRD-UV-ZA
SGRD-UV-ZB
SGRD-UV-ZS (2 copies)
SGRD-UV-RC (5 copies)
SGRD-UV-R (13 copies)
SGRD-UV-AI
SGRD-UV-D
SGRD-UV-P
SGRD-UV-V
SGRD-UV-Y
Aberdeen Proving Ground, MD 21010-5425 — 27

Department of Health and Human Services
National Institutes of Health
The National Library of Medicine
Serial Records Section
8600 Rockville Pike
Bethesda, MD 20894 — 1

Stemson Library
Academy of Health Sciences
Bldg 2840, Rm 106
Fort Sam Houston, TX 78234-6100 — 1

US Army Research Office
ATTN: Chemical and Biological Sciences Division
P.O. Box 12211
Research Triangle Park, NC 27709-2211 — 1

AFOSR/NL
Bldg 410, Rm A217
Bolling AFB, DC 20332 — 1

Commander
US Army Chemical Research, Development & Engineering Ctr
ATTN: SMCCR-MIS
Aberdeen Proving Ground, MD 21010-5423 — 1